

Molecular characterization of antibiotic resistant bacteria and assessment of antimicrobial residues in the dairy milk in India

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Introduction

- Food safety is a great concern in low- and middle-income countries. India is the largest milk producer in the world and milk is consumed by the most people.
- Antimicrobial resistance (AMR) is a serious threat to the health of both humans and animals, and resistant bacteria can also be spread through food
- Therefore, the risk of exposure to antibiotic resistant bacteria and antibiotic residues in milk needs serious attention.
- This study was designed to investigate the presence of AMR bacteria and antibiotic residues in milk in two states in India, Assam and Haryana.

Materials & Methods

- Milk samples were collected from two Indian states during 2016-18.
- 729 samples (285 from Assam and 444 from Haryana) were analyzed for microbiological studies
- 730 milk samples (330 from Assam and 400 from Haryana) were analyzed for antibiotic residues.

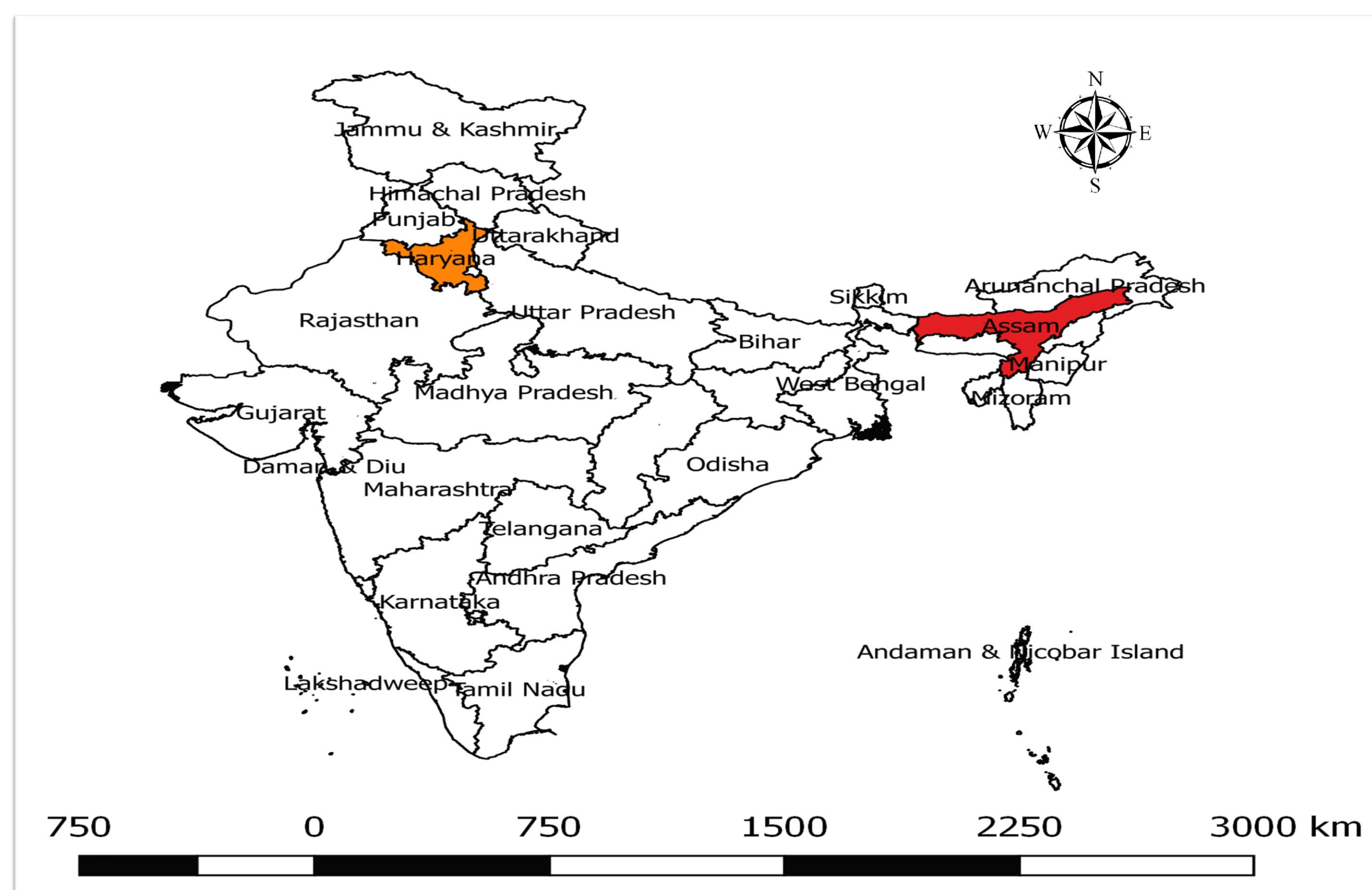
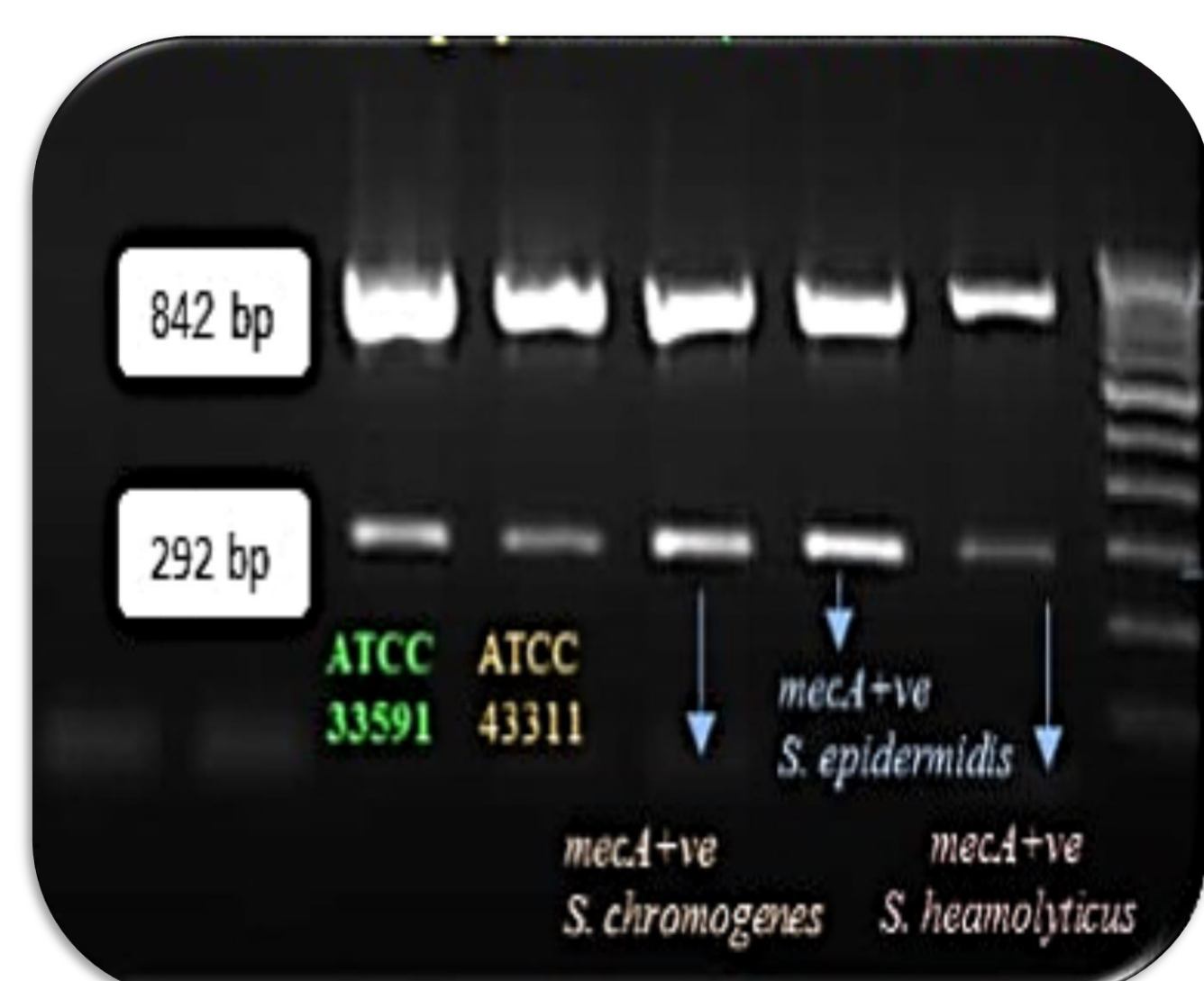
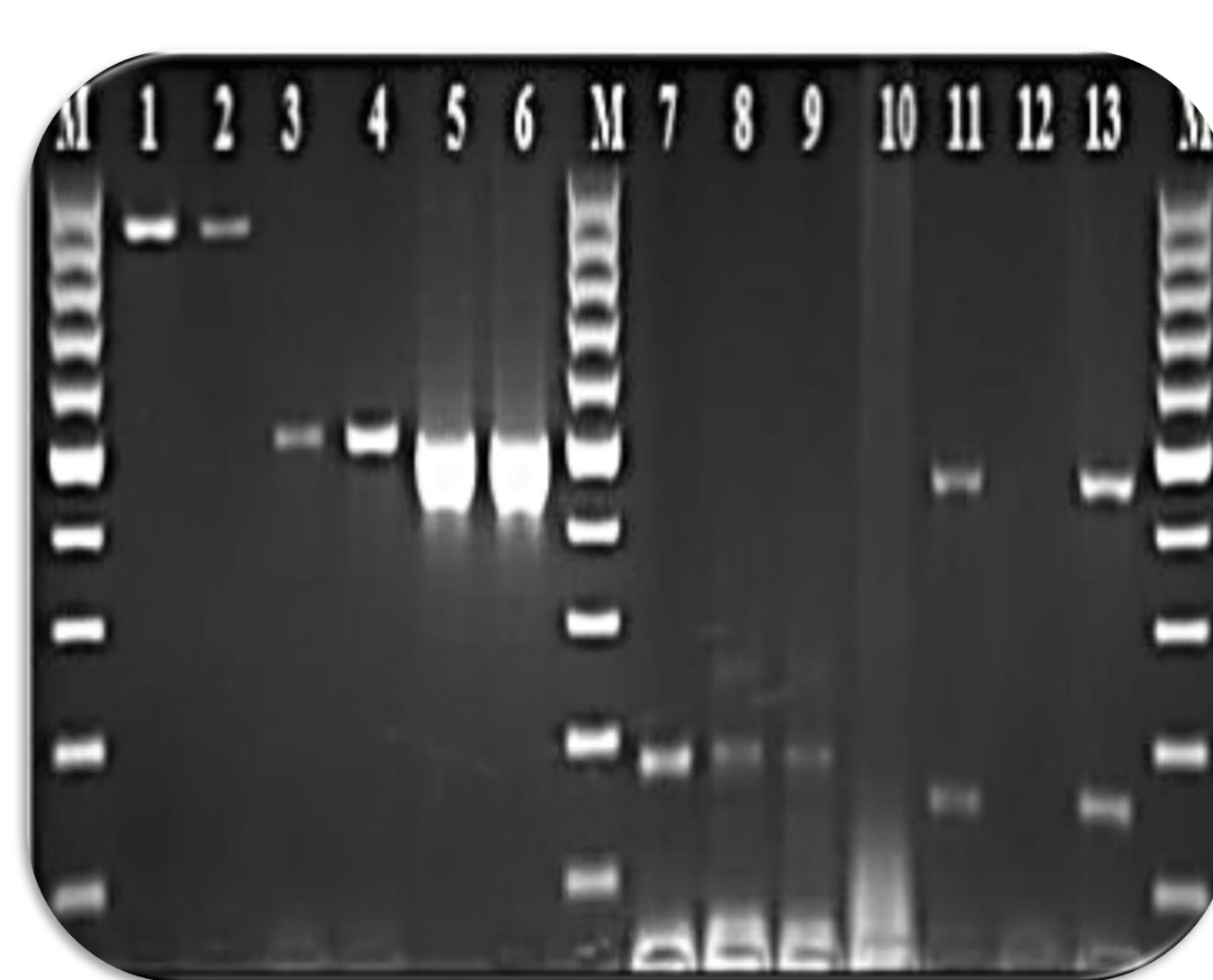


Fig 1: Map depicting the sampling states from India

- Bacteria were isolated as per standard protocol and subjected to antibiotic disc diffusion testing
- Gram-positive bacteria were tested against cefoxitin (30µg), methicillin (5µg) and oxacillin (1µg) for screening methicillin resistance
- Gram-negative bacteria were tested against cefoxitin (30µg), cefotetan (30µg), cefotaxime (30µg), ceftazidime (30µg), imipenem (10µg) and meropenem (10µg) was used for screening beta-lactamases (extended spectrum beta-lactamase, metallo-beta-lactamase, AmpC betalactamase).
- The isolates resistant by disc diffusion test were screened for methicillin resistance genes and beta-lactamases (ESBL, MBL, AmpC) genes using polymerase chain reaction
- The collected samples were also analyzed for antibiotic residue testing using spore-based assay and Charm Rosa test.



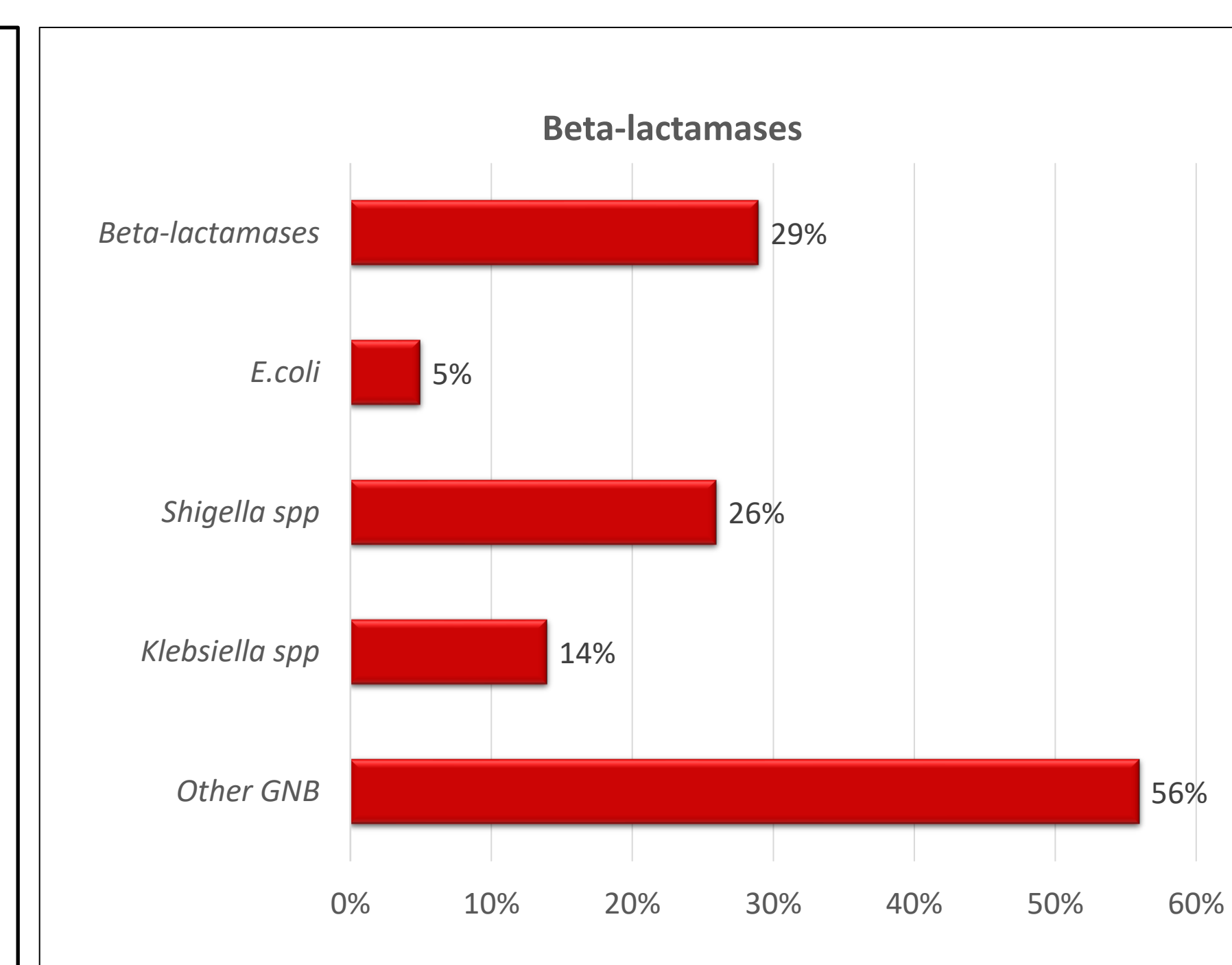
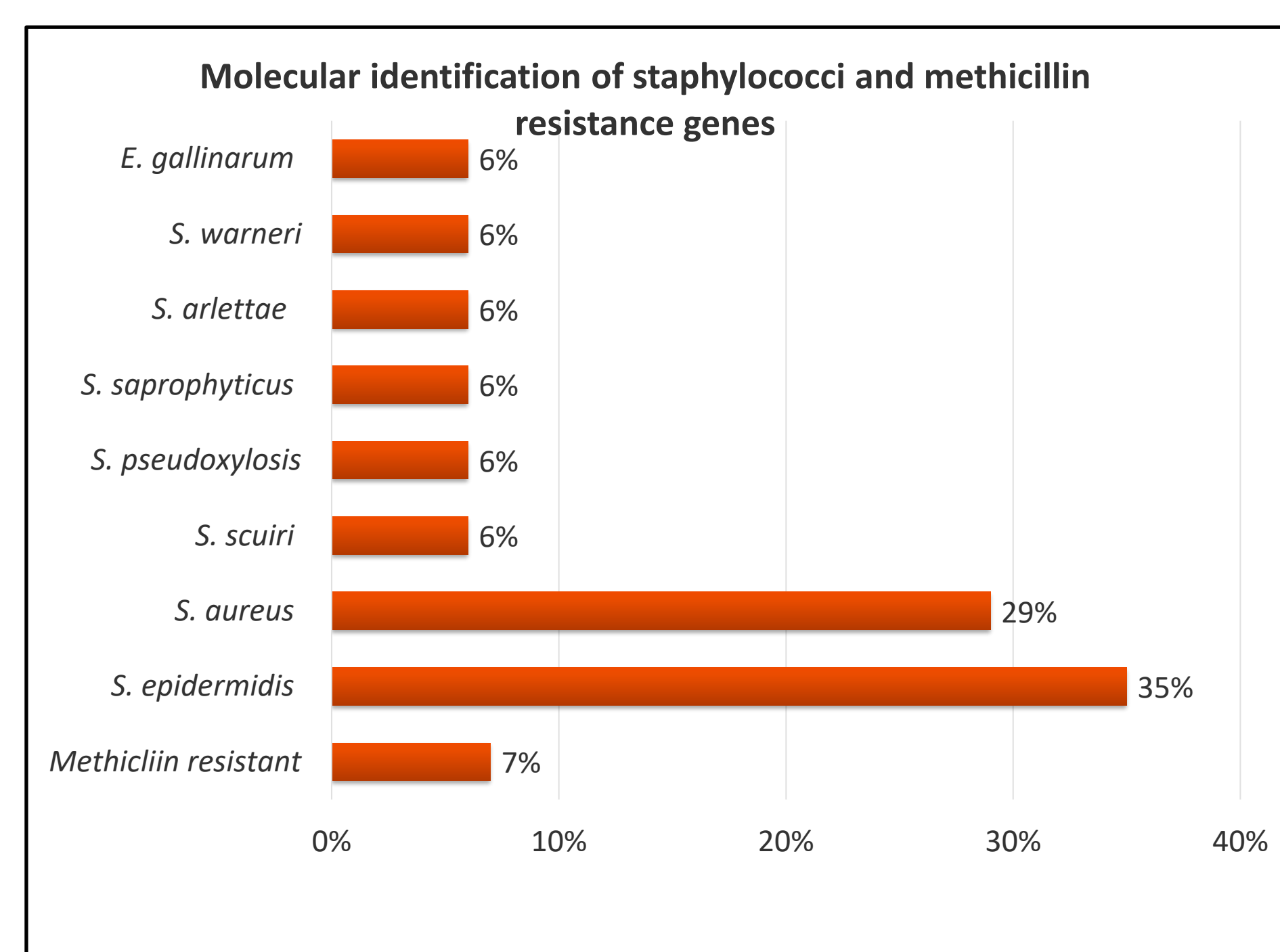
Methicillin resistance markers in genus *Staphylococcus* & *mecA* by duplex PCR



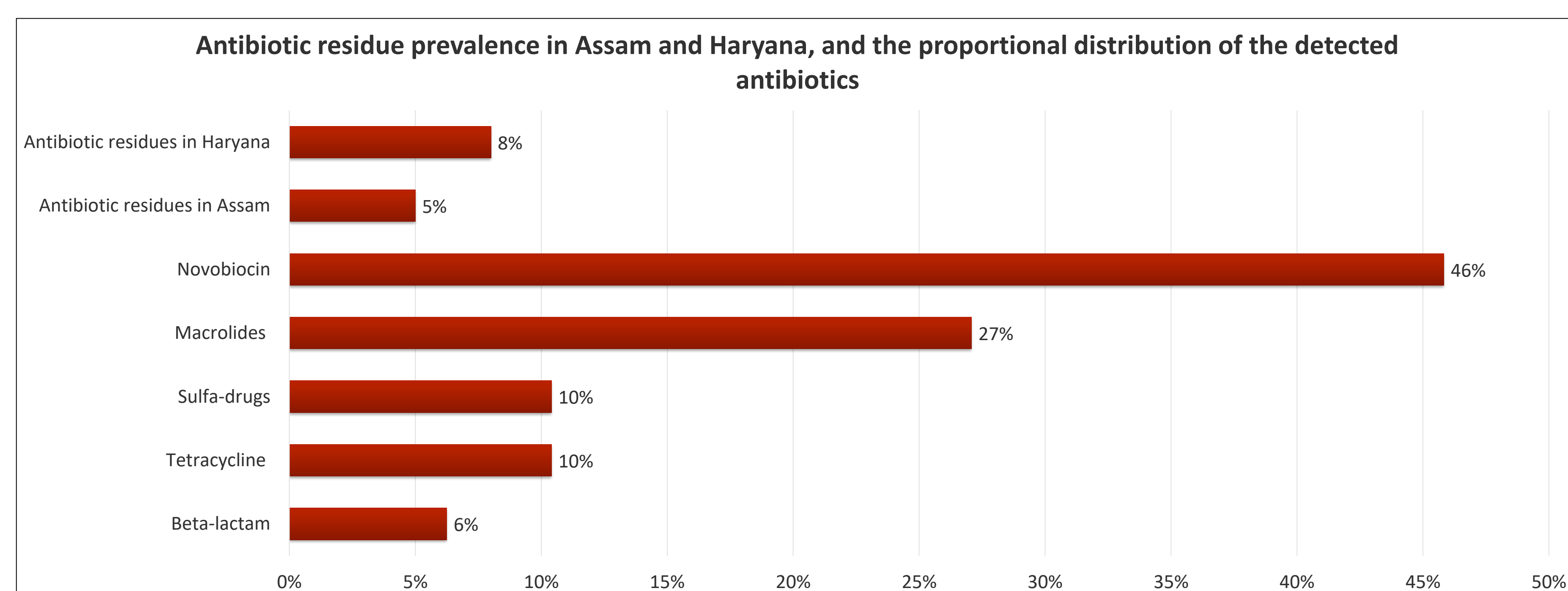
Detection of ESBL/MBL/AmpC genes in Gram-negative bacteria by mPCR

Results

- In total, 256 resistant Gram-positive isolates were obtained by disc diffusion testing, most were identified as genus *Staphylococcus*, and 7% (17/256) of phenotypically resistant staphylococci harbored methicillin resistance *mecA* / *mecC* genes by genotyping.
- The 17 methicillin resistant staphylococci identified, carried *mecA* (15) and *mecC* (2) genes.
- Of the resistant Gram-negative isolates (148) obtained by disc diffusion testing, by genotyping 29% (n=43/148) were beta-lactamase isolates among *E.coli*, *Klebsiella* spp., *Shigella* spp. and other gram-negative bacteria.
- ESBL* - 16% (7/43), *AmpC* - 60% (26/43), *MBL* - 14% (6/43).
- Some bacteria also had combinations of resistance genes: *MBL+AmpC* - 7% (3) and *ESBL+AmpC* - 2% (1).



- The prevalence of antibiotic residues in milk of Haryana was 8% (32/400) and in Assam was 5% (16/330).
- The antibiotic residues detected were novobiocin 46% (22/48), macrolides 27% (13/48), sulfa drugs 10% (5/48), tetracycline 10% (5/48), and beta-lactam 6% (3/48).



- Antibiotic resistant bacteria and residues were found in milk samples from both the states, but more commonly in Haryana compared to Assam.

Conclusions

- The study found both the occurrence of 7% methicillin resistance genes among isolated *Staphylococcus* spp, as well as 29% *ESBL*, *MBL* and *AmpC* producers among the isolated *E.coli*, *Klebsiella* and *Shigella* spp.
- Also, antibiotic residues were found in 7% of the milk samples.
- Since the milk sampled were all at the point of sales, these resistant bacteria could point to risks for human health, and even if the milk was boiled before consumption, the antibiotic residues could contribute to the risks for humans.
- Hence there is need for intensified awareness creation and surveillance on the occurrence of antimicrobial resistance and antibiotic residues in animals and food chain of animal origin to devise strategies for preventing their spread.

Acknowledgements

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